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Original Article

The inhibition effects of two species of Astragalus extracts on mushroom tyrosinase activity

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Abstract

Melanin pigment plays a critical role in camouflage and protection against harmful effects of solar radiation. Melanogenesis is under complex regulatory control by multiple agents. Tyrosinase is a multifunctional, which catalyzes the first two steps in mammalian melanogenesis. In this study, inhibitory effects of *Astragalus fasciculifolius* and *Astragalus gypsicolus* on diphenolase activity of mushroom tyrosinase were evaluated. .Ethanol 80% extracts were prepared for screening tests. The IC_{50}, K_i, Km and Vm values of *Astragalus fasciculifolius* and *Astragalus gypsicolus* were measured then compared them . We found that Both extracts show mixed type inhibition on mushroom tyrosinase when L-DOPA was used as a substrate.

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Introduction

Melanogenesis is a physiological process resulting in melanin production [1]. Melanin is one biopigment that wildly distributed in nature [2].It is responsible for skin, eyes and hair color in mammals and essential for wound healing, sclerotization of exoskeleton in insects. In addition, melanin is responsible of browning of vegetable and fruits during their handling and storage after harvest [3].

This process dependent on activity of melanogenic enzymes such as tyrosinase (EC.1.14.18.1) [4]. Another name of tyrosinase, which is also known as pholyphenol oxidase (ppo) [5]. PPO is a copper-containing glycoprotein wildly distributed in microorganisms, animals, plants and insects. This enzyme accepts many cathhochols and phenols as substrate [4].

Tyrosinase catalyses two steps of melanogenesis, the hydroxylation of monophenolic compounds to o-diphenols, monophenolase activity, and oxidation of the o-diphenols to o-quinones, diphenolase activity [6].

A common feature of all tyrosinase extracted from different sources in nature is that the central two copper-binding domain in active site. Each of the copper is connected to three histidines. Thus, three types of tyrosinase (oxy-, deoxy-, met-tyrosinase) due to different binuclear copper structures [7].

So far, a large number of tyrosinase inhibitors from both natural and synthetic sources have been identified. These inhibitors have various applications including: Agricultural, food fields, cosmetic and medicinal field [8].

For example the Kojic acid is a common tyrosinase inhibitor which is one of the metabolites produced by various bacterial or fungal strains such as penecillium and aspergillus [9]. Today, natural resources, for example, plants have an important role in the inhibition of tyrosinase. For example, recently some of the prenylated flavonoids such as norartocarpetin, artocarpesin and steppogenin were isolated from the *Artocarpus heterophyllus* and *Artocapus incisus* wood as antibrowning agents [10].

In this study, inhibitory effect of two native species of Khuzestan was evaluated on mushroom tyrosinase activity.

Materials and methods

Plants materials

Aerial organ of two species of Astragalus, *Astragalus fasciculifolius* and *Astragalus gypsicolus* were collected at Khuzestan, Iran in 2011. The plants were identified by the laboratory of pharmacognocy, Ahwaz Jundishapor University of Medical Sciences.

Chemicals

Mushroom tyrosinase (EC 1.14.18.1) was purchased from Sigma Company (USA). Kojic acid, DMSO and L-Dopa (Dihydroxy phenilalanin) were products of Aldrich Company (USA).

Extraction

The aerial part of *Astragalus fasciculifolius* and *Astragalus gypsicolus* were extracted by maceration method. Both species were dried, ground and stored in ethanol 80% for 72 hours. Filtered extracts were concentrated at 45ºC

temperature on a rotary evaporator and lyophilized. The extract obtained was stored in a refrigerator until used. 0.1 g of extracts were solved in 3 ml DMSO, then the yields were diluted with 25mM phosphate Buffer (pH: 6.8).

Enzyme assay of tyrosinase

The tyrosinase activity was determined according to Kubo and Kinst-Hori method with some modification. First 50 µl of tested sample (8.3-0.26 mg/ml) was mixed with 100 µl of mushroom tyrosinase (9.63 U/ml). After incubated at 25ºC for 5 min 100µl of 5 mM L-Dopa solution added to the mixture [11]. The amount of Dopachrom in reaction was immediately determined against blank in optical density at 475 nm in microplate reader (Tecan sunrise, Germany) during 35 min [12].

DMSO and Kojic acid were used as positive control. Inhibitory effects of the tested samples on the mushroom tyrosinase activity were expressed as % inhibition. IC_{50} values were defined as the concentration of inhibitor that inhibited 50% of tyrosinase activity under experimental conditions was named IC50 value [7].

Percent inhibition of tyrosinase activity was calculated as:

%Inhibition={[(A-B)-(C-D)]/(A-B)}×100

A: optical density at 475 nm without test sample

B: optical density at 475 nm without test sample and enzyme

C: optical density at 475 nm with test sample

D: optical density at 475 nm with test sample, but without enzyme

Measurment of kinetic parameters

100 µL of mushroom tyrosinase solution and different volume of L-Dopa (10-100 µl) and potassium phosphate buffer (pH: 6.8) with or without 50 µL of tested samples were added to a 96-well plate. Using a microplate reader, the initial rate of Dopachrome formation from the reaction mixture was determined by linear increase in absorbance at 475 nm. Kinetic parameters, Michaelis constant (Km) and maximal velocity (Vm) of the tyrosinase activity were determined using a Linewear-Burk plots. The inhibition constant (Ki) was measured by the dixon plots.

Results

In this study, inhibitory effects of *Astragalus fasciculifolius* and *Astragalus gypsicolus* on diphenolase activity of mushroom tyrosinase were evaluated, so L-Dopa is used as substrate of tyrosinase.

Both extracts showed antityrosinase activity weaker than Kojic acid. IC_{50} values of extracts *Astragalus fasciculifolius* and *Astragalus gypsicolus* are expressed 1.5127, 1.2117 mg/ml, respectively (Fig. 1).

The results indicated both extracts mixed-type inhibited tyrosinase activity. Linewear-Burk plots for inhibition of tyrosinase by Astragalus fasciculifolius and Astragalus gypsicolus are shown in Figure 2 &3. Kojic acid exhibited mixed-type of inhibition on tyrosinase.

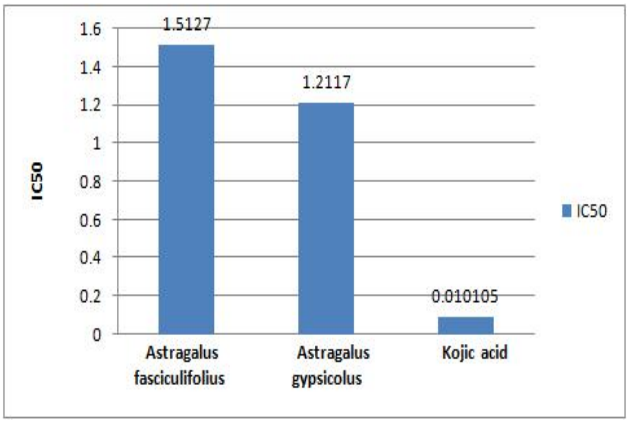

Figure 1. IC_{50} value for tested samples and positive control (Kojic acid).

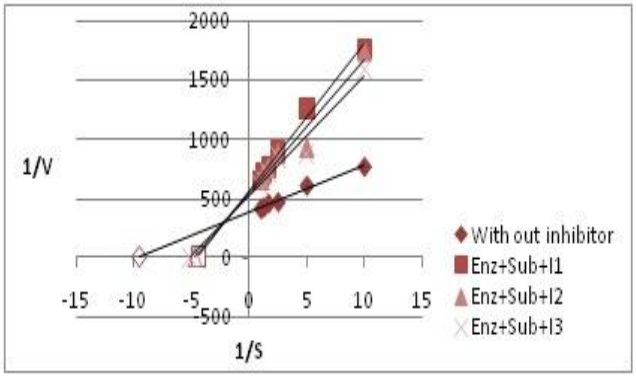

Figure 2. Lineweaver–Burk plot for inhibition of *Astragalus fasciculifolius* on mushroom tyrosinase for the catalysis of L-Dopa.

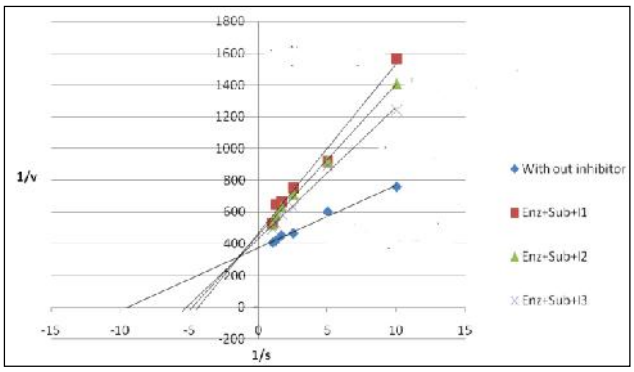

Figure 3. Lineweaver–Burk plot for inhibition of *Astragalus gypsicolus* on mushroom tyrosinase for the catalysis of L-Dopa.

So in their plots increased the Km and decreased the Vm value. In other words, they binded to the active site of enzyme. Km and Vm values are shown in Table1.

Table 1. Kinetic parameters in presence of extracts and Kojic acid

Name	K_m	V_{max}
Astragalus fassiculifolius	0.2108	0.00186
Astragalus gypsicolus	0.2085	0.002196
Non inhibitor	0.1815	0.002634
Kojic acid	0.2934	0.001879

The inhibition constant (K_i) of *Astragalus fasciculifolius* and *Astragalus gypsicolus* were estimated to be 1.549 and 1.2296 mg/ml, respectively.

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